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13. ABSTRACT (Maximum 200 Words)

c-myb, along with A-*myb* and B-*myb,* belongs to the *myb* gene family which codes for nuclear proteins that bind DNA in a sequence-specific manner and function as regulators of transcription. There is a large body of evidence to suggest a role for c-*myb* in breast development and cancer. c-*myb* is highly expressed in all estrogen receptor positive (ER+) breast tumors as well as ER+ mammary carcinoma cell lines. In addition, our *in situ* hybridization studies show that c-*myb* is expressed at high levels in ductal cells from breast tissues of virgin and pregnant mice. To address the role of c-*myb* in mammary development and cancer, we have created c-*myb* conditional knockout mice where the expression of this gene is interrupted specifically in the mammary gland using the Cre-*lox* system. To date, we have generated two female mice that are homozygous for the c-myb floxed alleles (conditional deletion alleles), and additionally, one of the two female mice bears the WAP-cre transgene while the other carries the MMTV-cre transgene. The generation of these breast-specific c-myb conditional knockout mice will afford us the opportunity to dissect the role of c-myb in normal breast development and cancer.

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Introduction

The development of breast cancer is a multistage process involving alterations in tumor suppressor genes and oncogenes. Overexpression of c-myb oncogene has been reported in human estrogen receptor positive (ER+) tumors and ER+ mammary carcinoma cell lines (1,2,3, also our unpublished data). In fact, cumulative data have shown c-myb to be one of the most frequently altered genes in breast cancer (1,4,5). In addition, recent data have shown a correlation between c-myb oncogene amplification and hereditary BRCA1 breast cancer (6). c-*myb,* along with A-*myb* and B-*myb,* belongs to the *myb* gene family which codes for nuclear proteins that bind DNA in a sequence-specific manner and function as regulators of transcription (7,8).

c-myb is predominantly expressed in hematopeoietic cells and its essential role for the proliferative potential of these cells has been well established (9). Homozygous null c-myb mutant mice die in utero due to defects in fetal hepatic hematopoiesis (10). However, the role of c-myb in breast development and breast cancer is beginning to emerge only recently. The first evidence that implicated a role for c-myb in breast tumors came from the observation that this gene is highly expressed in all estrogen receptor positive (ER+) breast tumors and mammary carcinoma cell lines (1,2,3, also our unpublished data). In addition, expression of a dominant negative mutant of the c-myb in ER+ breast carcinomas was found to result in their growth arrest and loss of tumorigenicity (our unpublished observations). To determine whether c-myb gene plays a role in breast development, we examined the pattern of expression of this gene in breast tissues derived from virgin, pregnant and lactacting mice. *In situ* hybridization studies show that c-myb is expressed at high levels in ductal cells derived from breast tissues of virgin and pregnant mice but is down-regulated in breast tissue of lactating mice. This observation combined with the observation that c-myb is highly expressed in ER+ breast tumor cells suggests that this gene might play a critical role in estrogen-mediated ductal cell proliferation.

Body

The main objective of my application is to study the effects of c-myb gene deletion on breast development by generating c-myb conditional knockout mice where the c-myb gene is deleted specifically in mammary gland using the embryonic stem (ES) cell technology and the Cre-*lox* system.

During the grant years 2000 to 2003, I had generated 4 karyotypically normal recombinant c-myb ES cell clones (R1/51, R1/62, R1/161 and RW4/23) from R1 ES and RW4 ES parental cells and our modified conditional c-myb targeting vector. From three of these recombinant clones, I was able to generate 7 karyotypically normal type II conditional c-myb deletion ES clones (R1/51.29/5, R1/51.18/57, R1/51.18/82, R1/161/91, RW4/23/47, RW4/23/62 and RW4/23/128). Three out of four type II conditional c-myb deletion ES cell clones (R1/51.29/5, R1/51.18/82, R1/161/91 but not RW4/23/47) that were sent out for microinjection into blastocysts, produced chimeras. Only chimeras obtained from R1/51.18/82 and R1/161/91 type II conditional c-myb deletion ES cell clones, produced c-myb$^{F/wt\ (F=floxed,wt=wild\text{-}type)}$

heterozygotes-gone germline. When I crossed the c-myb$^{F/wt}$ heterozygotes, I was able to obtain some homozygotes, c-myb$^{F/F}$,indicating that there were no splicing interference from the loxP's, flanking the c-myb region I want to delete.

To obtain breast-specific c-myb knockout mice, I crossed the c-myb$^{F/wt}$ heterozygotes with MMTV-cre mice to obtain F1 mice. I crossed the F1 mice (c-myb$^{F/wt}$ /MMTVcre+ or c-myb$^{F/wt}$ /MMTVcre-) with each other to obtain F2 breast-specific knockout mice (c-myb$^{F/F}$ /MMTVcre+). However, after 4 F2-litters and 34 mice, I did not obtain any c-myb$^{F/F}$/MMTVcre+ mice nor c-myb$^{F/F}$/MMTVcre- mice. Since there may be leakiness into the hematopoetic compartment associated with the MMTV promoter (11) and c-myb is critical for fetal hematopoesis (10), presumably, these c-myb$^{F/F}$/MMTVcre+ mice died in utero. To test if there is leakiness into the hematopoetic compartment, I prepared DNA from blood and mammary tissues of three c-myb/MMTVcre mice with various genotypes and performed Southern blot analysis (Figure 1). As seen in figure 1, There is leakiness into the blood and mammary tissues.

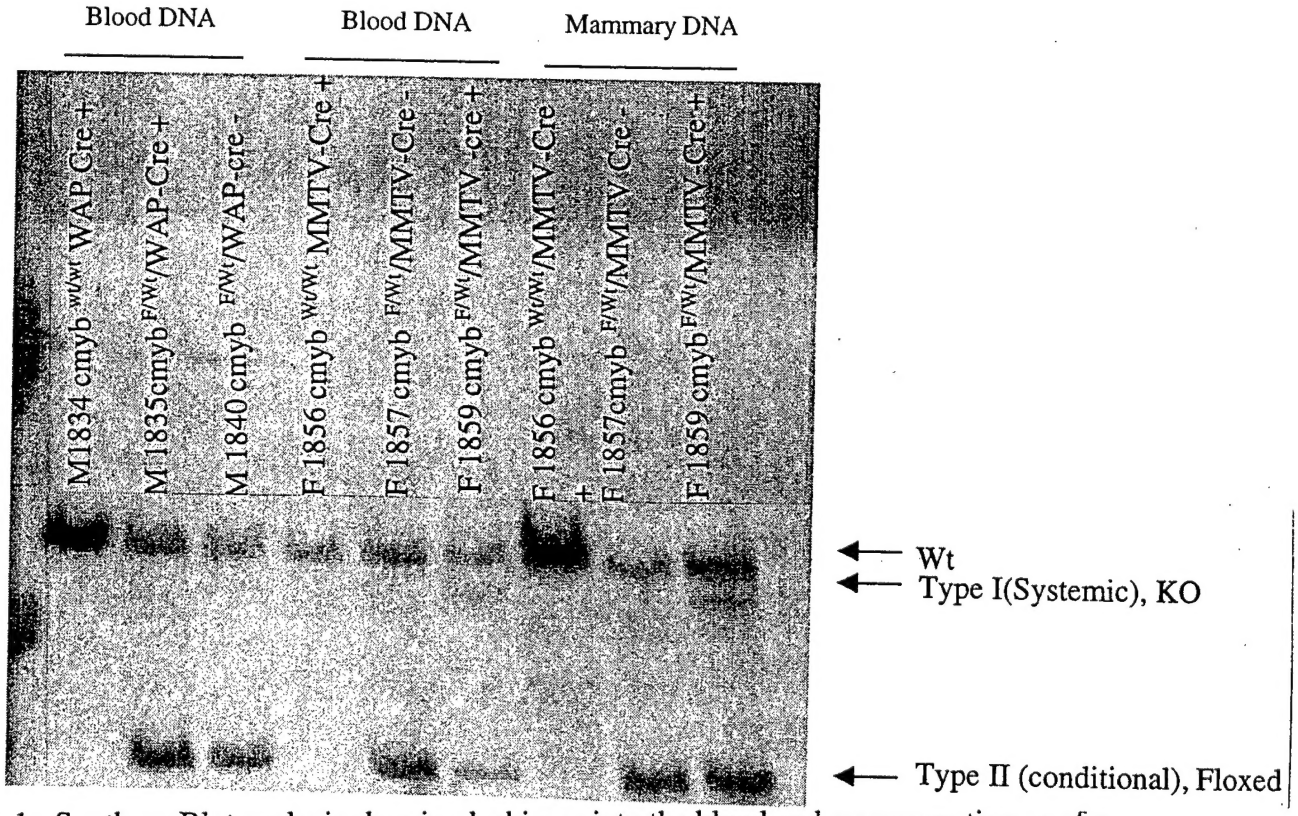

Figure 1: Southern Blot analysis showing leakiness into the blood and mammary tissues of c-myb$^{F/wt}$ /MMTVcre+ mouse but no leakiness into the blood of c-myb$^{F/wt}$ /WAPcre+ mouse.

To date, I have obtained a total of 79 c-myb/MMTVcre from 10 F2-litters. Figure 2 shows that I have one female breast-specific c-myb KO mouse, #2332 c-myb$^{F/F}$/MMTVcre+. Interestingly, #2332 displayed some sign of leakiness even in tail tissue. Apparently, the leakiness is not severe enough to cause lethality. A possibility as to why I did not obtain any c-myb$^{F/F}$/MMTVcre- mice may be a lower chance of obtaining these mice since both dam and sire are MMTVcre positive.

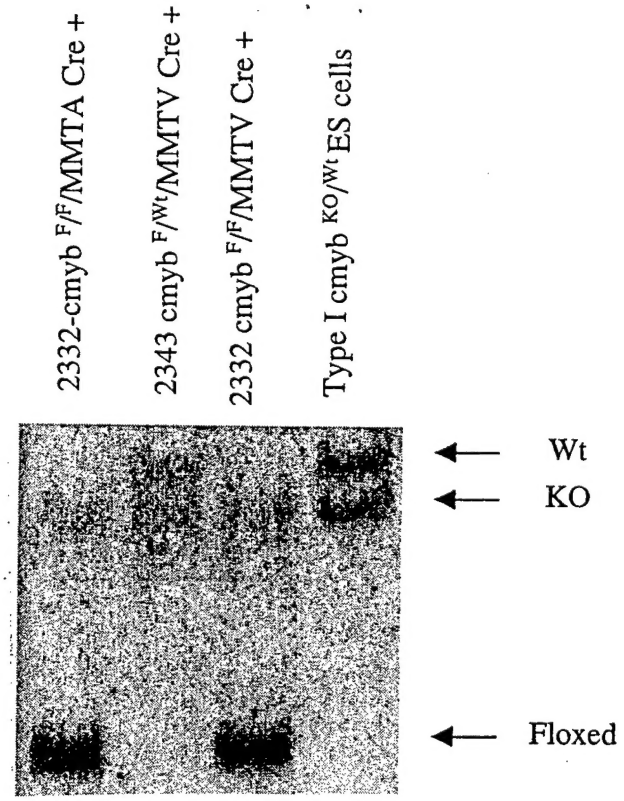

Figure 2: Southern Blot analysis on mouse tail DNA indicating the procurement of a breast-specific c-myb KO female mouse, #2332 c-myb$^{F/F}$ /MMTVcre+.

In addition to the MMTV mouse model, I have generated another breast-specific c-myb KO mouse model using WAPcre mice. So far, I have only one F2 c-myb/WAPcre litter with 7 pups. Figure 3 indicates that I have 3 c-myb$^{F/F}$ /WAPcre mice, #2552, #2553 and #2554. #2551 is a female that is negative for the WAPcre transgene. #2553 is a male that carries the WAPcre transgene. #2554 is WAPcre positive and is another breast-specific c-myb KO mouse model.

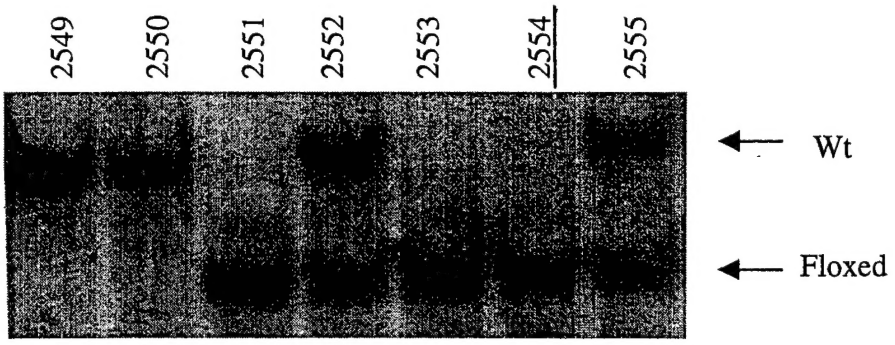

Figure 3: Southern blot analysis of mouse tail DNA from F2 c-myb/WAPcre pups.

Key Research Accomplishments

- Production of 7 karyotypically normal type II conditional c-myb deletion ES cell clones.
- Creation of c-myb floxed mice.
- Generation of breast-specific c-myb KO mouse models:

  -c-myb$^{F/F}$ /MMTVcre+

  - c-myb$^{F/F}$ /WAPcre+.

Reportable Outcomes

None

Conclusions

I have generate c-myb floxed (c-myb^{F}) mice which will be an important model not only for breast development and breast cancer but also for examining the role c-myb in the normal development and cancer progression of other tissues as well, such as blood, colon and brain. By generating two breast-specific KO mouse models, I have completed the major goal of my original grant proposal. We believe these breast-specific c-myb conditional knockout mice will provide two invaluable models for dissecting the role of c-myb in normal development as well as gain insight into the role of its aberrant expression in breast cancer. Furthermore, we believe that a detailed molecular understanding of how c-myb contributes to tumor progression is of major importance for future therapy.

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